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Severe Thrombocytopenia in Patients Treated With Suramin: Evidence for an Immune Mechanism in One

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Although suramin has long been used to treat human trypanosomiasis, recent clinical trials have tested its efficacy against the acquired immunodeficiency syndrome (AIDS) and various malignancies. Thrombocytopenia was observed in early trials with suramin in AIDS, but has been uncommon in patients treated for solid tumors. Here we describe 5 patients out of a total of 67 (7%) who developed severe thrombocytopenia while receiving suramin as part of a phase II clinical trial for metastatic prostate carcinoma refractory to hormonal therapy. IgG purified from one patient's plasma caused suramin-dependent platelet aggregation. There was also evidence of crossreactivity between suramin and heparin in this system. An immune mechanism, however, could not be documented in the other cases, suggesting that multiple mechanisms may be responsible for severe thrombocytopenia in this patient population. © 1996 Wiley-Liss, Inc.*

Key words: suramin, thrombocytopenia, immune

INTRODUCTION

Suramin is a polysulfonated naphthylurea that has been in use for the treatment of human trypanosomiasis since the 1920's. More recently, it has been shown to have antiretroviral and tumoricidal activity and is currently being tested in a variety of therapeutic trials. Unfortunately, the drug also has a number of adverse effects including hematologic toxicity [1,2], adrenal insufficiency [3], peripheral neuropathy [4], fatigue, anaphylactoid reactions [5], maculopapular skin eruptions [6], and acute renal insufficiency [7]. In early trials in the treatment of acquired immunodeficiency syndrome (AIDS), suramin caused neutropenia in 26% of patients and thrombocytopenia (<50,000/μl) in 12% [8]. However, when suramin was given to patients with solid tumors, platelet counts of <50,000/μl were not described [9,10].

Although most investigators have assumed that the mechanism by which suramin causes cytopenias is marrow suppression, Seidman et al. [11] described a patient with severe thrombocytopenia associated with suramin that appeared to be immune-mediated. We now report on 5 patients who developed severe thrombocytopenia among a series of 67 who received suramin as a part of a phase II clinical trial for metastatic prostate carcinoma refractory to hormonal therapy. In one of these patients there was evidence that the thrombocytopenia was mediated by an immune mechanism.

All patients received suramin initially as five daily intravenous infusions. Subsequent dosing was adjusted to maintain the peak suramin plasma concentration at 300 μg/ml and the trough at 175 μg/ml. Patients also received 20 mg of hydrocortisone each morning and 10 mg each evening, as well as 250 mg of aminoglutethimide four times daily beginning on the first day of treatment. The protocol was approved by the National Cancer Institute's Institutional Review Board, and all patients gave written informed consent prior to participating in the study.

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CASE REPORTS

Patient 1 was a 54-year-old man with stage D2 prostate carcinoma. When he began treatment with suramin, his platelet count was 253,000/μl, his white blood cell (WBC) count was 7,500/μl, and his hemoglobin was 11 g/dl. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were normal. He had previously been treated with depot leuprolide, which was continued throughout the study. After five daily doses of suramin (Mobay Pharmaceutical Co., New York, NY), the platelet count was 213,000/μl. The mean trough suramin plasma concentration during this period was 145.7 ± 32.1 μg/ml (range, 102–182 μg/ml), and the mean peak was 380.2 ± 52.4 μg/ml (range, 290–433 μg/ml).

Three days later the platelet count was 19,000/μl. During this time the patient received no additional medication, including no heparin. Over the next 2 days the platelet count fell to 3,000/μl, when the patient developed epistaxis. The WBC count also fell to 2,100/μl over the initial 3 days with a nadir of 1,400/μl, 6 days after the last dose of suramin, but rose to 2,900/μl the next day. Hemoglobin dropped to 7.7 g/dl with the epistaxis. PT was 13.9 sec (normal, 9.4–13.7 sec), APTT was 35.9 sec (normal, 22.5–34.8 sec), and the fibrinogen concentration was 546 mg/dl (normal, 160–345 mg/dl). There were no schistocytes observed on a peripheral blood smear.

The patient was given 8 units of platelets, but 30 min later his platelet count was only 4,000/μl. He then began methylprednisolone 30 mg every 12 hr for 10 days, and a 2-day course of intravenous gammaglobulin, 1 g/kg/day. Because of continued severe epistaxis the patient received 4 units of packed red blood cells and 7 separate transfusions of random donor platelets, totaling 59 units over the first 4 days. Although there was no history of prior transfusions and a screen for human leukocyte antigen (HLA) antibodies was negative, the highest platelet count reached over this period was 17,000/μl. The patient's platelet count remained <20,000/μl for a total of 13 days. An attempted bone marrow aspiration was unsuccessful, but a marrow biopsy revealed complete replacement with metastatic carcinoma.

Nineteen days after the last dose of suramin the patient's platelet count had risen to 75,000/μl, and his WBC count was normal. One month later the platelet count was 106,000/μl.

Review of the records of the other 66 patients treated with the same protocol revealed 4 additional cases of severe, acute thrombocytopenia. The data for these are summarized in Table I. Patient 2, who had a clinical course very similar to that of patient 1, underwent a bone marrow aspiration and biopsy which showed adequate megakaryocytes, but this was performed just as his thrombocytopenia was resolving. Patients 3–5 did not have bone marrow examinations. Anemia, attributable to gastrointestinal bleeding, occurred only in patient 3. None of the patients in Table I became neutropenic.

MATERIALS AND METHODS

IgG Purification

IgG was purified from normal plasma and the plasma from all 5 patients by passage over a column of protein G sepparose (Pharmacia Biotech, Piscataway, NJ). Suramin was removed from IgG by passage over diethylaminoethyl (DEAE) sepharose (Pharmacia Biotech). Suramin was undetectable (<5 μg/ml) in the final IgG preparations.

Suramin Assay Method

Plasma suramin concentrations were determined by the method of Supko and Malspeis [12].

Platelet Aggregation Studies

Platelet aggregation studies were performed with plasma collected from patients 1 and 2 during the time of their severe thrombocytopenia. Equal volumes of each patient's plasma (anticoagulated with 0.32% sodium citrate) and platelet-rich plasma from a normal donor were mixed at 37°C in a Chrono-Log Aggregometer (Chron-Log Corporation, Havertown, PA) and observed for spontaneous aggregation. After 10 min, suramin was added in various concentrations, and the platelet suspension was observed for aggregation for an additional 10–15 min. The same concentrations of suramin were also added to platelet-rich plasma diluted 1:1 with normal plasma rather than the patients' plasma. In some experiments heparin was substituted for suramin in order to test for crossreactivity with other molecules bearing a high negative-charge density.

Similar studies were conducted with mixtures of suramin-free IgG (final concentration, 7.5 mg/dl) from the plasma of patient 1 and equal volumes of platelet suspension.

^{3}H-Serotonin Release Assay

Serotonin release assays were performed with purified, suramin-free IgG from all 5 patients. Because suramin is extensively bound to plasma proteins [13], the effect of a given dose is highly dependent upon the protein environment. Therefore, platelet-rich plasma, rather than suspensions of washed platelets which are typically used in other serotonin-release assays [14], was used in order to duplicate the milieu used in platelet aggregation studies with IgG and to approximate the clinically-achieved concentrations of suramin. Platelet-dense granules were radiolabeled by incubating normal whole blood with 10 nmol/l ^{3}H-serotonin (New England Nuclear, Boston, MA) for 30 min at 37°C. Platelet-rich plasma was prepared

TABLE I. Summary of Clinical Data for Patients 2–5

	Patient			
	2	3	4	5
Onset of thrombocytopenia	Day 7	Day 10	Day 7	Day 10
Nadir platelet count (per μl)	6,000	21,000	10,000	9,000
Initial recovery (after last dose)	14 days	3 days	4 days	7 days
Evidence of disseminated intravascular coagulation (DIC)	No	No	No	No
Signs of infection	No	Yes	Yes	No
Rechallenge with suramin	No	No	No	Yes[a]
Serotonin-release assay	Negative	Negative	Negative	Negative

[a]Thrombocytopenia did not recur.

and mixed with an equal volume of buffer (0.1 M NaCl, 0.05 M tris, pH 7.35) containing normal or patient IgG (final concentration, 7.5 mg/ml) and variable amounts of suramin (final concentration, 0–1,000 μg/ml).

After rocking at room temperature for 1 hr, the platelet suspensions were diluted with 10 ml of cold (4°C) buffer, filtered through polycarbonate filters (0.4-μm pores; Nucleopore Corporation, Pleasanton, CA), and washed with an additional 10 ml of cold buffer. The filters were then dissolved in NCS Tissue Solubilizer (Amersham Corporation, Arlington, IL), neutralized with glacial acetic acid, and added to Econoflour (New England Nuclear) for liquid scintillation counting.

RESULTS

Platelet Aggregation Studies

When plasma from patient 1 (containing ~100 μg/ml suramin) was added to an equal volume of normal platelet-rich plasma, aggregation tracing was superimposable on the result obtained with normal platelet-rich plasma alone (Fig. 1, bottom). As suramin concentration was increased in successive studies, platelet aggregation developed and increased with suramin doses up to 1,300 μg/ml (Fig. 1, top) Suramin also caused platelet aggregation in the absence of the patient's plasma, but the response was always less (Fig. 1, middle and top).

Although suramin-free IgG from patient 1 did not stimulate platelet aggregation, addition of 250 or 375 μg/ml of suramin caused a response (Fig. 2).

When plasma from patient 2 was substituted in the platelet aggregation assays, no aggregation occurred beyond that observed with normal plasma and suramin.

To determine whether other highly-charged molecules could substitute for suramin in stimulating platelet aggregation, 0.2 units/ml of heparin were added to a mixture of normal platelets and plasma from patient 1 (final suramin concentration, 50 μg/ml). Aggregation rate and extent

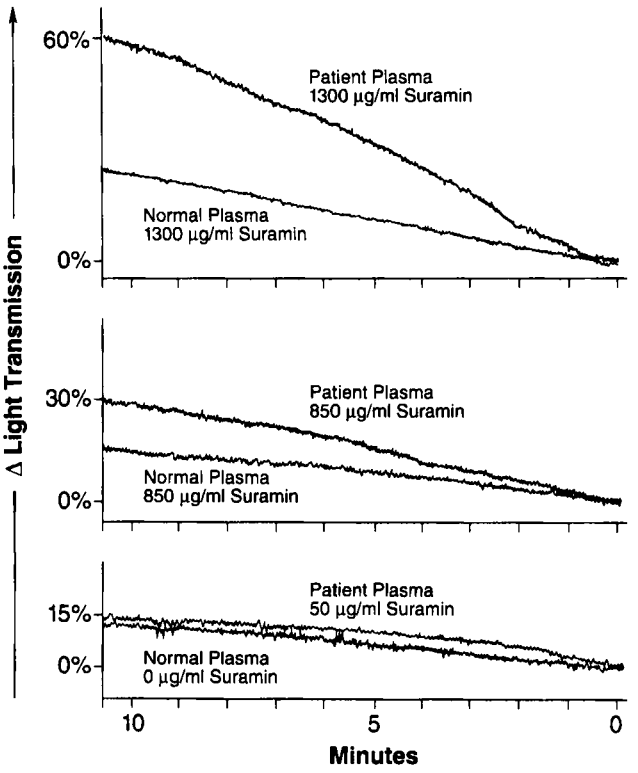

Fig. 1. Platelet aggregation tracings obtained by mixing equal volumes of normal platelet-rich plasma and plasma from the same normal donor or plasma from patient 1 in the presence of the concentrations of suramin indicated.

increased (Fig. 3). However, enhancement was lower when 0.02 or 2 units/ml of heparin was added.

^{3}H-Serotonin Release Studies

At suramin concentrations >400 μg/ml, labeled platelets consistently released ^{3}H-serotonin in the presence of normal IgG (Fig. 4). However, with platelets from 2 of 4 normal donors, partial release occurred at 250 μg/ml

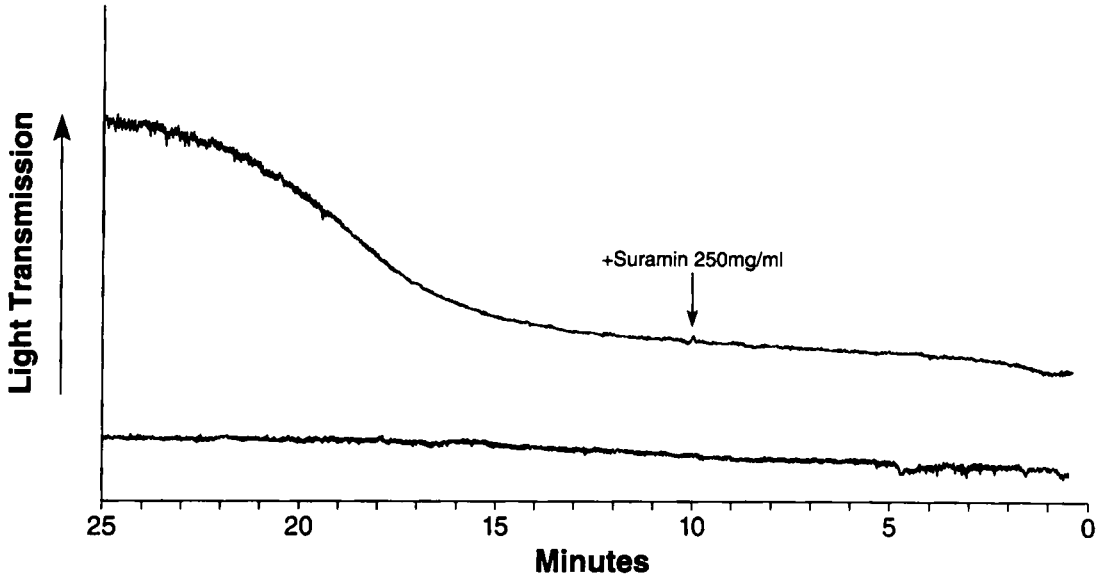

Fig. 2. Platelet aggregation tracings obtained by mixing equal volumes of normal platelet-rich plasma and a suramin-free solution of IgG (15 mg/ml) purified from patient 1. Lower tracing was obtained in the absence of suramin. In upper tracing, 250 μg/ml suramin was added at the time indicated.

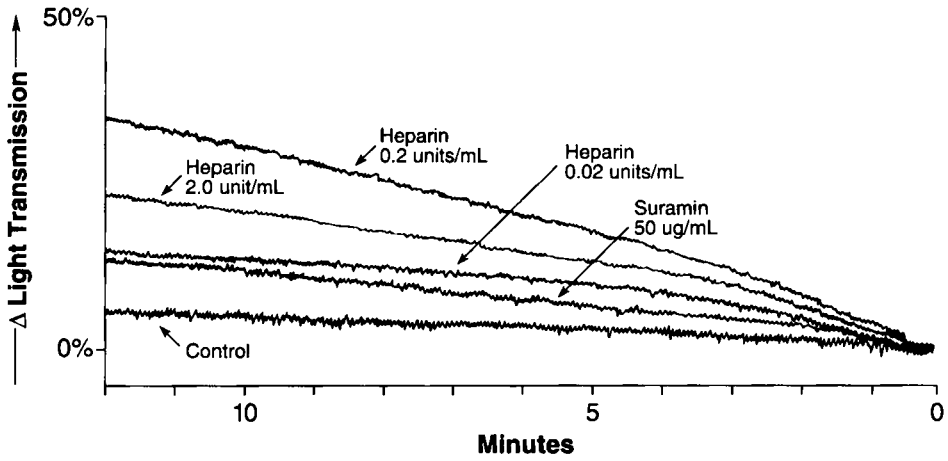

Fig. 3. Platelet aggregation tracings obtained by mixing equal volumes of normal platelet-rich plasma and plasma from patient 1. All mixtures contained 50 μg/ml suramin. Variable heparin concentrations are indicated. Control contained only normal plasma without suramin or heparin.

suramin in the presence of IgG from patient 1 (Fig. 4). This increased sensitivity was not observed with IgG from any of the other 4 patients, even with donor platelets that had given a positive result with patient 1.

DISCUSSION

While suramin-related thrombocytopenia has been previously described, it has usually been attributed to marrow suppression [15]. Seidman et al., however, reported a patient whose severe thrombocytopenia developed so rapidly that accelerated peripheral platelet destruction was the more likely cause [11]. Seidman et al. hypothesized an immune mechanism on the basis of an increase in platelet-associated IgG, although this finding is nonspecific [16].

Patient 1 is clinically very similar to the one reported by Seidman et al. [11]. Although our patient's bone marrow was infiltrated with carcinoma, he was not thrombo-

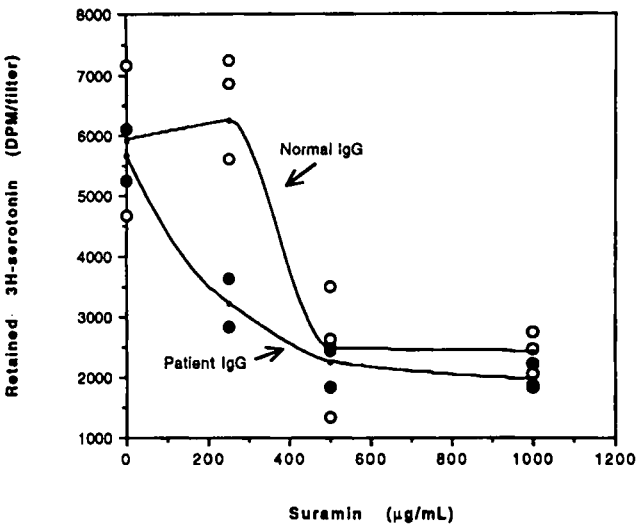

Fig. 4. ^{3}H-serotonin release assays performed with normal IgG and suramin-free IgG purified from the plasma of patient 1. Vertical axis represents ^{3}H-serotonin (disintegrations per minute [DPM]/filter) retained by the platelets after 1 hr of mixing with an equal volume of IgG and the suramin concentrations indicated.

cytopenic until the second week of treatment, when the plasma suramin level was 100–200 μg/ml. Severe thrombocytopenia then developed too abruptly to be attributable to marrow failure alone. Furthermore, there was no evidence of other causes of such a clinical event, such as disseminated intravascular coagulation or acute infection.

In addition to the clinical features suggesting immune-mediated thrombocytopenia, we gained some laboratory support for this diagnosis. Although suramin stimulated platelets in normal plasma, in the presence of plasma or of purified IgG from patient 1, the aggregation response was significantly greater (Figs. 1 and 2), and serotonin release occurred at a lower suramin concentration (Fig. 4).

Including 0.2 units/ml heparin in the platelet suspension increased the rate of aggregation, suggesting a degree of crossreactivity between suramin and heparin in this system (Fig. 3). Therefore, patients with suramin-related thrombocytopenia probably should not be exposed to heparin unless a drug-dependent immune mechanism has been ruled out. Kelton et al. [17] have reported similar crossreactivity between heparin and other large, highly-charged compounds in diagnostic tests for heparin-induced thrombocytopenia. The fact that higher (2 units/ml) and lower (0.02 unit/ml) concentrations of heparin augmented the aggregation response less than an intermediate concentration is again consistent with the formation of immune complexes dependent upon a critical concentration of polyanion [14,17]. Unfortunately, the role of immune complexes in the thrombocytopenia seen in patient 1 could not be investigated further because of insufficient plasma samples.

On reviewing the records of all 67 patients who had received suramin on the same protocol, we found 4 other cases of acute, severe thrombocytopenia (Table I). Although one of these (patient 2) had a clinical course closely resembling the case we have described, efforts to document an immune mechanism (platelet aggregation and serotonin-release studies performed with the patient's IgG) failed.

The clinical presentations of 2 other patients suggested acute infection, and the fourth patient was rechallenged with suramin without a recurrence of thrombocytopenia. Sufficient plasma samples were available from the latter 3 cases only to permit purification of their IgG and testing in the serotonin-release assay. In all cases their IgG failed to increase platelet sensitivity to suramin.

Of the 5 severely thrombocytopenic patients, only patient 1 was simultaneously neutropenic. However, among 170 patients treated with suramin for metastatic prostate carcinoma at our institution (on the protocol described here as well as on other protocols), 5 other patients developed severe neutropenia (mean count, 62/μl) without thrombocytopenia (N. Dawson, personal communication). Therefore, the relationship of the two cytopenias in our patient 1 in unclear.

Aminoglutethimide, which has been described to cause thrombocytopenia, was administered as part of the protocol to all patients, and must be considered as a potential offending agent. However, only 12 cases of neutropenia or thrombocytopenia were observed in one series of 1,333 patients receiving the drug [18]. Furthermore, the in vitro data suggesting a suramin-dependent immune thrombocytopenia in patient 1 make it unlikely that aminoglutethimide was involved.

CONCLUSIONS

Overall it appears that severe thrombocytopenia in patients receiving suramin is not uncommon, occurring in 5 of 67 patients (7%) treated on a single protocol at our institution. However, the mechanisms for the thrombocytopenia must vary and may not always be related to the drug. Patients receiving suramin typically have advanced malignancies and are subject to multiple causes of severe thrombocytopenia, such as acute infection and disseminated intravascular coagulation. Our data suggest, however, that suramin-dependent, immune-mediated thrombocytopenia must now also be included in the differential diagnosis of thrombocytopenia in these patients.

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